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Yield Loss Caused by *Meloidogyne graminicola* on Lowland Rainfed Rice in Bangladesh[1]

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Abstract: The impact of *Meloidogyne graminicola* on growth and yield of lowland rainfed rice was assessed with and without carbofuran in a rice-wheat rotation area of northwestern Bangladesh. The experiment was conducted on farmer fields and at a research station, with experimental plots arranged in a randomized complete block design. Prior to transplanting, rice seedling height and dry weight were greater ($P \leq 0.05$) and soil levels of *M. graminicola* were lower ($P \leq 0.05$) in the treated seedbed plots compared to the nontreated control plots. Nematicide application to the field at transplanting had a greater effect ($P \leq 0.05$) on mid-season plant growth than did nematicide application to the seedbed at sowing, and rice yield increased by 1.0 t/ha where carbofuran was applied to the seedbed and field—both at the research station ($P \leq 0.05$) and on farmer fields ($P \leq 0.10$)—compared to a nontreated control. This is the first report of a negative impact of *M. graminicola* on growth and yield of lowland rainfed rice in production fields in Bangladesh.
Key words: Bangladesh, carbofuran, *Meloidogyne graminicola,* nematode, rice, wheat.

Infestations of the rice root-knot nematode, *Meloidogyne graminicola* Golden and Birchfield, have been reported in a range of rice production systems in South and Southeast Asia, including upland, irrigated, lowland rainfed, and deepwater rice (Arayarungsarit, 1987; Bridge, 1990; Bridge and Page, 1982; Cuc and Prot, 1992; Gaur et al., 1993, 1996; Miah et al., 1985; Mondal et al., 1988; Netscher and Erlan, 1993; Prot and Matias, 1995; Roy, 1987; Sharma et al., 2001). Despite the ubiquity of *M. graminicola* in Asian rice production, estimates of yield loss under natural infestations are few and have been limited to upland rice systems (Arayarungsarit, 1987; Netscher and Erlan, 1993). There have been no field investigations on yield loss caused by *M. graminicola* for rice subjected to varying durations of soil flooding, as occurs under lowland rainfed production.

Studies conducted in controlled environments with artificial inoculation conditions have addressed important questions of how water management affects *M. graminicola* activity. These studies demonstrated that yield loss is greatest under nonflooded conditions (Plowright and Bridge, 1990; Prot and Matias, 1995; Soriano et al., 2000; Tandingan et al., 1996) and that early-season flooding can reduce *M. graminicola* damage on rice (Rao and Israel, 1971; Soriano et al., 2000). However, field-based investigations are needed to best relate these estimated yield losses to that which occurs in infested production fields.

In Bangladesh, *M. graminicola* has been most often associated with deepwater and pre-monsoon upland rice systems (Bridge and Page, 1982; Miah et al., 1985), whereas only minor infestations have been reported in lowland rainfed rice areas (Miah et al., 1985). However, severe infestations of *M. graminicola* were recently identified using a soil bioassay test for several rice nursery seedbeds and production fields in northwestern Bangladesh (Padgham, 2003), where the dominant cropping system is lowland rainfed rice rotated with wheat. This study was undertaken to assess the impact of *M. graminicola* infestations on plant growth and yield of lowland rainfed rice in rice-wheat production fields.

Materials and Methods

A yield loss experiment was conducted from May through November 2001 at two locations in northwestern Bangladesh. One of the locations was at the Bangladesh Rice Research Institute's Regional Station in Rajshahi (24° 3′N latitude and 38° 2′E longitude). A second location was a farmer production area in the Natore district, approximately 30 km southeast of the research station. Both of these locations were found to be infested with *M. graminicola* in a regional survey carried out in May and June 2001 (Padgham, 2003). The experiment was arranged as a 2 × 2 factorial in a randomized complete block design, with a main factor of plus or minus nematicide treatment of soil in field plots, and a second factor of plus or minus nematicide treatment of soil in seedbed plots. On the research station, there were 6 replications of field plots and 12 replications of seedbed plots located in an *M. graminicola*-infested field. At the farmer location, there were five replications of field plots established across five fields, and seven replications of seedbed plots established across seven seedbeds.

Seedbed treatments: Rice seedbed plots at the research station were established in an *M. graminicola*-infested production field in mid-June. Soil was leveled in 24

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1-m^2 plots, and 10-cm-high earthen barriers were constructed around each plot to prevent movement of carbofuran between plots. One day later, half of the plots were treated with carbofuran (Furadan 5G, Aventis Crop Science India Ltd.) at 2 kg a.i./ha and the other half were left untreated, for a total of 12 replications with each containing a nematicide-amended and non-amended treatment. The nematicide was mixed with granular fertilizer and broadcast evenly over the plot and then incorporated into the soil to a depth of 5 to 7 cm using a hoe. All seedbed treatments at the research station received urea, triplesuperphospate, muriate of potash, and gypsum fertilizers at 20, 40, 20, and 10 kg/ha of N, P_2O_5, K_2O, and S, respectively. Two days after nematicide application, plots were sown with 3-day-old germinated rice seed (cv. BR11) at 100 g seed/m^2. Plots were watered daily for the first 10 days and then as needed. Sixteen days after seeding, an additional 10 kg N/ha as urea was applied to all plots. Iprodione (Rovral 50 WP, 500 g/kg, Aventis Corp.) was applied to all plots at 18 days after seeding for control of brown spot disease.

At the farmer field location, experimental plots treated with 0 and 2 kg a.i./ha carbofuran were established on seven *M. graminicola*-infested farmer seedbeds. Each plot measured 4 m^2. The procedure for carbofuran application was the same as described for the research station. Urea was broadcast over the seedbed plots at 30 kg N/ha 1 week after sowing with BR11 rice seed. No other fertilizer was applied in accordance with typical farmer practice.

The effect of carbofuran application on the soil density of *M. graminicola* J2 and root-galling severity of rice seedlings were assessed at 3 to 4 weeks after sowing at both locations. At the research station, three subsamples containing soil and rice seedling roots were collected from each plot to 10-cm depth and composited. Nine subsamples were collected per farmer seedbed using the same procedure. Densities of *M. graminicola* J2 were determined by two 3-day pie pan extractions (Hooper, 1990) per composite sample. Roots from the remainder of the sample were removed and galling severity evaluated on a scale of 1 to 9. A rating of 1 indicates no visible galling; ratings of 2 to 9 indicate 1–3%, 4–10%, 11–25%, 26–35%, 36–50%, 51–65%, 66–80%, and >80% of the root system galled, respectively (Viaene and Abawi, 1996).

The effect of carbofuran application on seedling growth was assessed just prior to transplanting, which occurred at 27 days at the research station and at 41 days at the farmer site. Clusters of 10 seedlings were cut just below the soil surface from each plot for determination of shoot height and dry weight. On the research station seedbeds, three subsamples per treatment plot were collected from 4 of the 12 replications. Nine subsamples were collected per treatment plot on each of the farmer seedbeds.

Field treatments: At the research station, 30-day-old seedlings from the plus and minus carbofuran seedbed plots were transplanted into main field plots measuring 1 m × 1.4 m and treated with 0 or 2 kg a.i./ha of carbofuran. The four treatments were replicated six times and arranged in a randomized complete block design. At the farmer field location, eight initial experimental sites were established in five fields, with rice seedlings coming from five corresponding farmer seedbeds treated or not treated with carbofuran. Seedlings from the plus and minus seedbed treatments were transplanted into main field plots measuring 9 m^2 and treated with 0 or 2 kg a.i./ha of carbofuran. The average distance between each of the farmer sites was greater than 200 meters. Subsequent plant growth and nematode data were recorded from only five of the eight farmer sites, as the three remaining sites were severely damaged by rats.

Fertilizer applications in the main fields were the same for both locations and were done according to recommendations for commercial production of BR11 rice (Bangladesh Rice Research Institute, 2000). Additionally, fields were scouted weekly for signs of foliar pest damage or disease. Between maximum tillering and booting stage of rice development, all fields were sprayed with phosphamidon (Diamacron 100L, Novartis Corp., Mumbai, India), a non-carbamate pesticide, at 1.6 ml/liter of water, for control of stem borer infestations.

Soil and plant sampling: The effect of carbofuran on populations of *M. graminicola* in soil was assessed at maximum tillering and at harvest using a rice-seedling bioassay of soil from the experimental plots. At the experiment station, soil samples were taken at a 10-cm depth with a 3-cm-diam. push probe, inserted next to 20 rice hills in each plot. Because of difficulties encountered in extracting soil from flooded plots with a push probe, sampling of the farmer fields was done with a trowel to a 10-cm depth. A composite soil sample was placed in pots and seeded with BR11 rice for the bioassay. The rice seedlings were watered twice daily and maintained outdoors in ambient temperatures. After 30 days, rice roots from all bioassay tests were washed and rated for root-galling severity on a 1-to-9 scale, as described previously. The plot sampling and bioassay procedure was repeated at harvest. Soil from all plots was sampled with a push probe, and the bioassay was conducted for 23 days. A 23-day soil bioassay with wheat seedlings was also conducted at the end of the rice season to assess potential root-knot nematode damage on wheat in a rice-wheat rotation.

Plant height and number of tillers per hill were recorded at maximum tillering for both locations. Ten hills were sampled per plot in an 'X' pattern. At the end of the season, yield, number of panicles per plant, number of filled spikelets per panicle, percentage sterility, and thousand grain weight were recorded on all plots at the research station.

Data analysis: The pre-transplant seedbed data were analyzed using a one-way analysis of variance. Mid- and late-season data for the main effects and interactions of carbofuran application to the seedbed and field were analyzed using a general linear model for analysis of variance. Differences between individual treatment means were separated with a Tukey's significance test. Prior to analysis of variance, data for soil J2 populations were subjected to a log transformation and data for root-galling severity were subjected to a square-root arcsign transformation to stabilize the variance. All statistical tests were performed using Minitab, v. 13.1.

Results

Seedbed treatments: Seedling height and shoot dry weight of rice grown in the carbofuran-treated seedbed plots were greater ($P \leq 0.05$) than those of seedlings grown in the nontreated seedbed plots at both the research station and farmer field locations (Table 1). The number of *M. graminicola* J2 and the root-galling severity of rice seedlings were lower ($P \leq 0.05$) in the carbofuran-treated plots compared to those of the nontreated plots.

Field treatments: At both locations, the number of tillers per hill at mid-season was greater ($P \leq 0.05$) for the two treatments where carbofuran was applied to the field at transplanting as compared with the nontreated control (Fig. 1A). Mid-season plant height was greater ($P \leq 0.05$) for both carbofuran-amended field treatments at the research station, and for one of the two carbofuran-amended field treatments at the farmer location, compared to the nontreated control (Fig. 1B). Mid-season nematode populations at the research station were lower for the treatment that received carbofuran at seeding and transplanting, and for the treatment that received carbofuran at seeding only, compared to the nontreated control ($P \leq 0.05$) (Fig. 2A).

At the research station, rice yields increased by 0.2, 0.7, and 1.0 t/ha where carbofuran was applied to the seedbed only, to the field only, and to both the seedbed and field, respectively, compared with the nontreated control (Fig. 1C). Differences in rice yields between the control and the treatment that received nematicide at both seeding and transplanting were significant at $P \leq 0.05$. Yield loss occurred through a reduction in the number of panicles per plant ($P \leq 0.05$) (Table 2). Averaged across the five harvested farmer field plots, rice yields increased by 0.6, 0.6, and 1.0 t/ha with carbofuran application to the seedbed only, to the field only, and to both the seedbed and field, respectively, compared to the control. At this location, differences in rice yields between the control and the treatment that received nematicide at both seeding and transplanting were significant at $P \leq 0.10$.

Root-galling severity ratings of rice seedlings in the late-season bioassay ranged from 7.7 to 8.3 at the research station and from 6.2 to 8.0 at the farmer field location (Fig. 2B). Averaged over all of the four treatments, root-galling severity of rice seedlings increased from 5.3 to 8.1 between mid- and late season at the research station, and from 4.4 to 7.1 over the same period in the farmer fields. The late-season bioassay was terminated after 23 days due to poor seedling growth caused by nematode damage. In the bioassay with wheat, differences in root-galling severity were detected ($P \leq 0.05$) between plots previously treated with carbofuran at transplanting of rice compared with those not treated at transplanting (Table 3).

TABLE 1. Seedling growth of rice and soil nematode populations as affected by *M. graminicola,* and its control with carbofuran (C) in the seedbed.

Treatment	Shoot height (cm)	Shoot dry wt. (g)	J2/50 cm^3 soil[c]	RGS[d]
Research station[a]				
+ C	23.1	0.7	0	1.0
− C	17.3	0.4	41.33	2.9
	($P \leq 0.001$)	($P \leq 0.05$)	($P \leq 0.01$)	($P \leq 0.001$)
Farmer fields[b]				
+ C	29.1	1.2	56.1	1.0
− C	23.5	0.7	278.9	2.5
	($P \leq 0.05$)	($P \leq 0.05$)	($P \leq 0.05$)	($P \leq 0.05$)

[a] Mean of four replications.
[b] Mean of seven replications.
[c] J2 counts were log-transformed prior to analysis of variance. The J2 counts presented in this table are from pretransformed data.
[d] Root-galling severity was rated on a scale of 1 to 9. A rating of 1 indicates no visible galling; ratings of 2 to 9 indicate 1–3%, 4–10%, 11–25%, 26–35%, 36–50%, 51–65%, 66–80%, and >80% of the root system galled, respectively.

Discussion

Results of this investigation demonstrated that *M. graminicola* is capable of infection and substantial growth retardation of rice seedlings in nursery seedbeds. The increase in rice seedling height resulting from soil treatment with carbofuran was similar to that previously reported for rice seedlings treated with carbofuran for control of *M. incognita* (Fademi, 1987). The greater response to carbofuran application measured in the farmer seedbeds relative to that measured on the research station seedbeds was partly a function of different sampling times (41 days in the farmer seedbeds compared with 27 days at the research station).

Despite the improvement in rice seedling growth, the presence or absence of carbofuran application to the field plots at transplanting had a greater influence on mid-season plant growth and yield than did the residual effect from carbofuran application to the seedbed. The efficacy of carbofuran on *M. graminicola* was reported to decline 20 days after application in paddy rice (Krishna-Prasad and Rao, 1982); thus, the lack of a residual seedbed effect is not unexpected. However, any beneficial

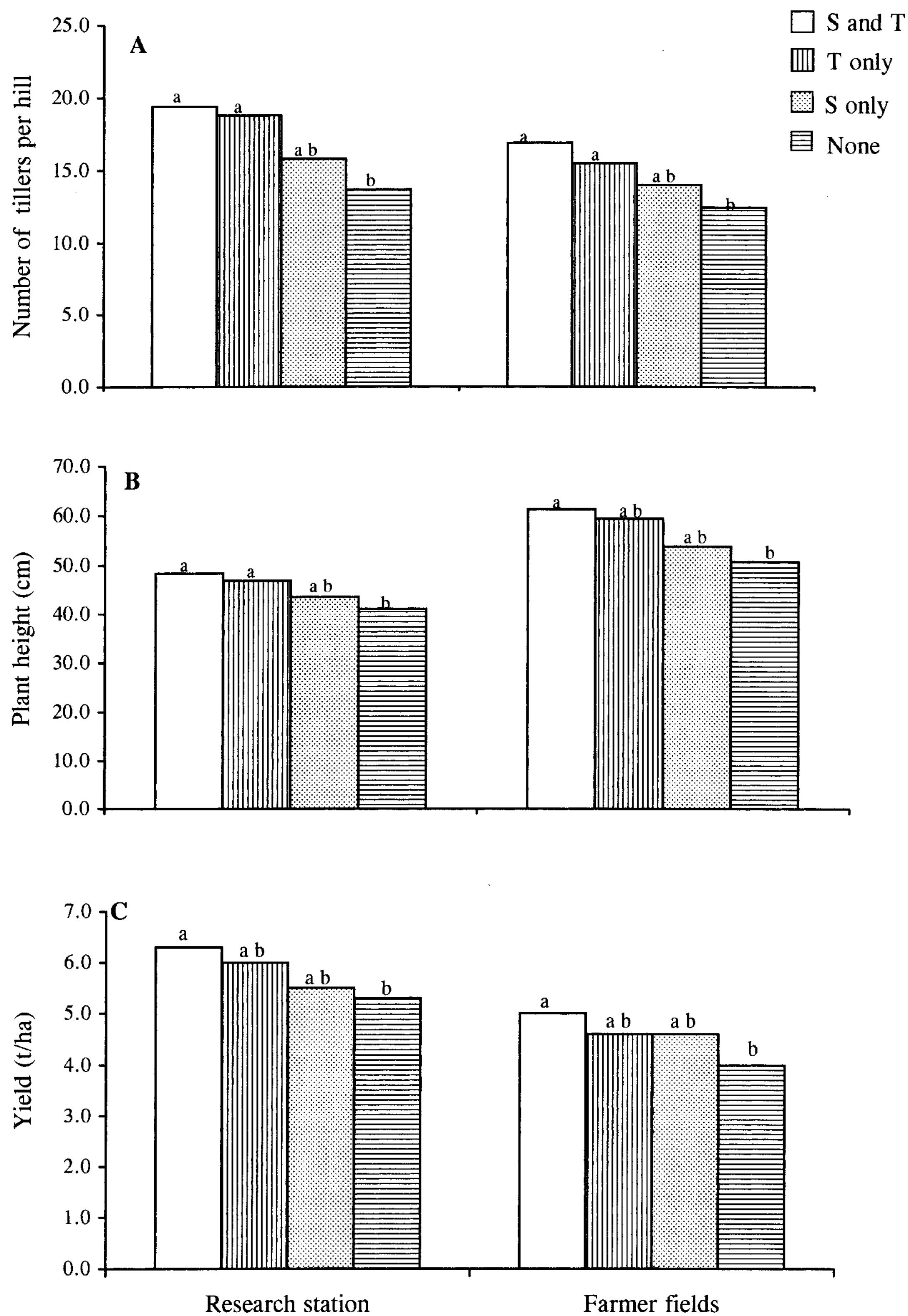

Fig. 1. Effect of carbofuran application at seeding (S) and transplanting (T) on mid-season tiller production (A) and plant height (B), and rice yield (C) in *Meloidogyne graminicola*-infested production fields. Bars with the same letter are not different at $P = 0.05$, as determined by a Tukey's test, except for Farmer field data in Figure C where bars with the same letter are not different at $P = 0.10$. Data for the research station is the mean of six replications, whereas the data from the farmer fields is the mean of five replications.

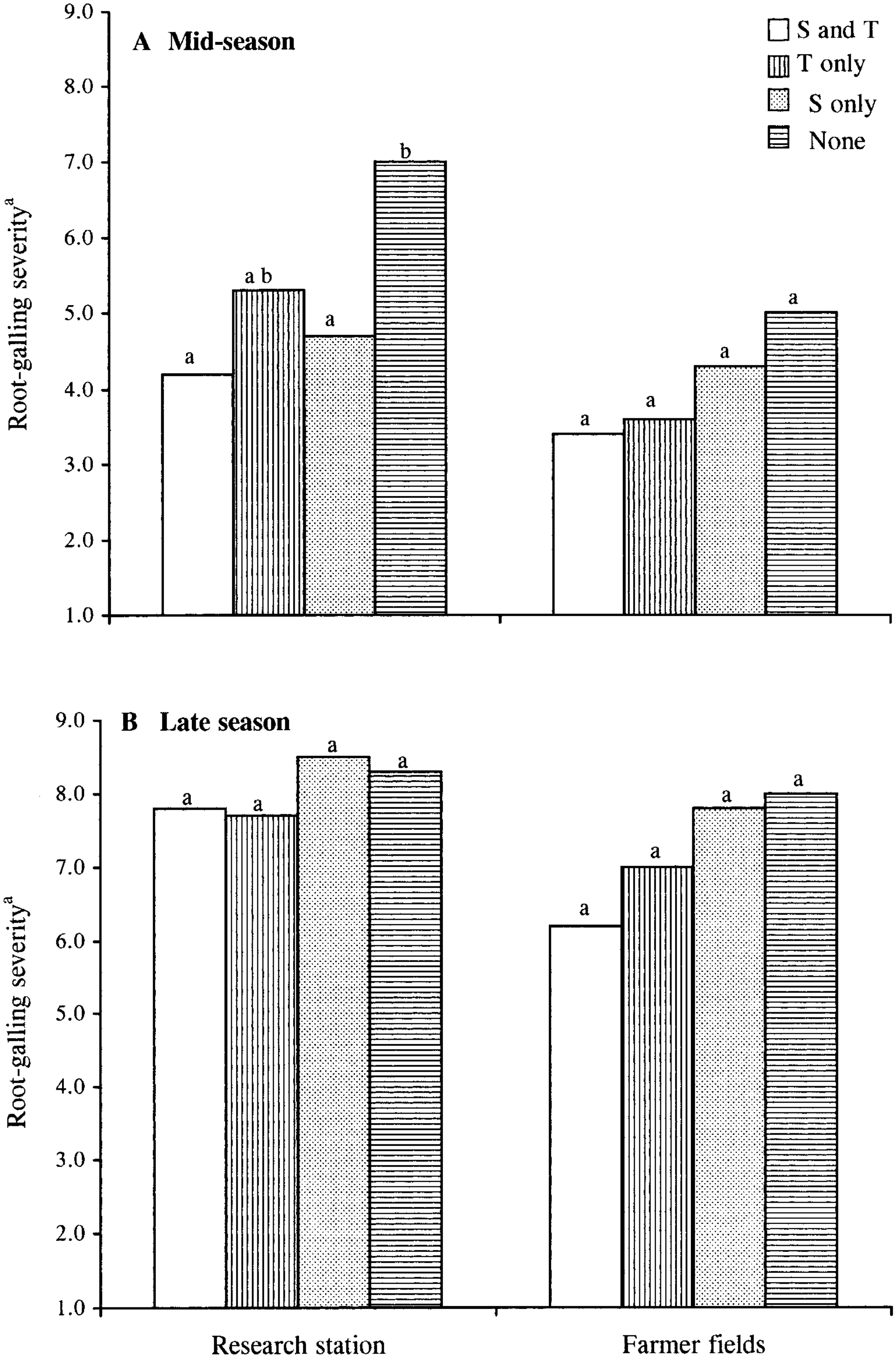

FIG. 2. Effect of carbofuran application at seeding (S) and transplanting (T) on soil populations of *Meloidogyne graminicola* as determined by a bioassay at mid-(A) and late (B) season. Bars with the same letter are not different at $P = 0.05$, as determined by a Tukey's test. [a]Root-galling severity rated on a scale of 1 to 9. A rating of 1 indicates no visible galling; ratings of 2 to 9 indicate 1–3%, 4–10%, 11–25%, 26–35%, 36–50%, 51–65%, 66–80%, and >80% of root galling, respectively. Data for the research station is the mean of six replications, whereas the data from the farmer fields is the mean of five replications.

TABLE 2. Yield component parameters as affected by application of carbofuran at seeding (S) and transplanting (T) at the research station.

Treatment	Panicles (per plant)	Filled spikelets (per panicle)	% Sterility	1,000 grain wt (g)
S and T	10.3 b	105.5 a	23.0 a	23.5 a
T only	9.4 a	103.9 a	23.2 a	23.9 a
S only	8.9 a	103.4 a	26.1 a	23.7 a
None	9.1 a	104.3 a	25.5 a	23.6 a

Mean of six replications. Values followed by the same letter in a column are not different at $P \leq 0.05$, as determined by Tukey's test.

effect resulting from *M. graminicola* control in the seedbed could be enhanced by sustained flooding of soil directly after transplanting, which would prevent any new infection of rice roots (Bridge and Page, 1982).

Treatment differences for mid-season root-galling severity were greater in the research station plots than those measured at the farmer field location. The use of different soil sampling devices (a push probe at the research station and a trowel at the farmer sites) may have influenced these results. Although the soil was sampled to the same depth with both instruments, the push probe extracted a uniform volume of soil with depth, while samples taken with a trowel contained a larger proportion of soil from the top few centimeters and may have, therefore, contained fewer nematode-infected roots.

In the bioassay with wheat, root-galling severity ratings as high as 6.0 were observed in the plots that received no nematicide in the preceding rice season. Infections of this severity could exacerbate the effect of abiotic stress factors, such as high ambient temperatures, associated with wheat production in Bangladesh. Damage to wheat has also been reported for populations of *M. graminicola* from India and Pakistan (Gaur and Sharma, 1999; Soomro and Hague, 1992), though no field studies have been conducted to estimate this nematode's impact on wheat under production conditions.

TABLE 3. Root-galling severity of wheat tested in a late-season bioassay of experimental plots as affected by prior application of carbofuran to rice at seeding (S) and transplanting (T).

	Research station[b]		Farmer fields[c]	
Treatment	RGS[a]	Range	RGS[a]	Range
S and T	3.5 a	2–5	3.7 a b	3–4
T only	3.7 a b	3–5	3.0 a	2–4
S only	4.8 a b	3–6	5.3 b	5–6
None	5.0 b	4–6	4.3 a b	3–5

At each location, values followed by the same letter in a column are not different at $P \leq 0.05$, as determined by Tukey's test.

[a] Root-galling severity rated on a scale of 1 to 9. A rating of 1 indicates no visible galling; ratings of 2 to 9 indicate 1–3%, 4–10%, 11–25%, 26–35%, 36–50%, 51–65%, 66–80%, and >80% of the root system galled, respectively.

[b] Mean of six replications.

[c] Mean of three replications.

The 1.0-t/ha increase in rice yield for the mean of plots where *M. graminicola* was controlled, both in the seedbed and main field compared to the nontreated control represented a 16% and 20% yield increase at the research station and farmer field locations, respectively. A late-season rat infestation on three of the eight farmer field plots reduced the number of observations (harvestable plots) by 40%. This reduction in the number of observations, coupled with the inherent variability between farmer field sites, made it difficult to detect yield and nematode population differences at that location.

The yield increase resulting from carbofuran application to both the seedbed and field was similar to the 18% rice yield increase reported when carbofuran was applied to control *Hirschmanniella oryzae* infestations in rice seedbeds and fields in India (Dabur et al., 1980). The results of this study were also within the range of the 12% to 33% rice yield increase obtained with nematicide application to *M. graminicola*-infested upland rice fields in Thailand (Arayarungsarit, 1987) but were lower than the 28% and 87% yield losses attributed to *M. graminicola* infestations of upland rice fields in Indonesia (Netscher and Erlan, 1993). The yield difference between nematicide-treated and nontreated plots measured in this study was less than that reported for estimates of yield loss under controlled conditions. *Meloidogyne graminicola* caused yield losses of 11% to 73% in simulations of intermittently flooded rice (Soriano et al., 2000), whereas under simulated upland conditions, yield loss from *M. graminicola* were between 20% and 98% (Plowright and Bridge, 1990; Prot and Matias, 1995; Tandingan et al., 1996).

The timing and duration of drought conditions in lowland rainfed rice influence the damage potential of *M. graminicola*. In August 2001, there was a post-transplant drought over much of Bangladesh. While the study area escaped the worst effects of the drought, farmer fields were not flooded for about 2 to 3 weeks during the vegetative growth stage and smaller intermittent droughts occurred later in the season. Nonflooded conditions such as these are favorable for *M. graminicola* invasion of rice roots (Bridge and Page, 1982), and *M. graminicola* infection during vegetative growth of rice can result in greater yield loss than would occur from nematode infection after booting and flowering stages (Soriano et al., 2000). To ensure similar growing environments between the two locations, the soil water status in the research station plots was maintained in such as way as to simulate soil water conditions observed on the farmer field plots.

To our knowledge, this is the first study to measure the impact of *M. graminicola* on yield of lowland rainfed rice in a rice-wheat production area of Bangladesh, and is one of only a few studies conducted on *M. graminicola* under natural infestations. The prevalence of intermittent drought during the rice season in northwestern

Bangladesh is characteristic of other rice-wheat producing areas of the Indo-Gangetic Plains (Gill, 1994; Zeigler, 1999). Thus, the results obtained in this study may be applicable to other rice-wheat areas of South Asia where *M. graminicola* has been reported. This study demonstrated that *M. graminicola* is negatively impacting plant growth in seedbeds and fields and is reducing rice yields under lowland rainfed conditions. This study also determined that control of *M. graminicola* in the field is more effective than its control in the seedbed for transplanted rice systems.

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